# IDENTIFICATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR *(BDNF) VAL66MET* GENE POLYMORPHISM AS A BIOMARKER ON COGNITIVE PERFORMANCE OF AGING BRAIN IN RESPONSE TO AN 8-WEEK OF EXERGAMES TRAINING

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# IDENTIFICATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR *(BDNF) VAL66MET* GENE POLYMORPHISM AS A BIOMARKER ON COGNITIVE PERFORMANCE OF AGING BRAIN IN RESPONSE TO AN 8-WEEK OF EXERGAMES TRAINING

**by**

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## LIST OF SYMBOLS

| | |
|---|---|
| Aβ | amyloid beta |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AACD | Aging-Associated Cognitive Decline |
| ACC | Anterior Cingulate Cortex |
| ACh | Acetylcholine |
| AD | Alzheimer's Disease |
| ADAS-Cog | Alzheimer's Disease Assessment Scale-Cognitive Subscale |
| aMCI | amnestic Mild Cognitive Impairment |
| AMDI | Advanced Medical and Dental Institute |
| ApoE | Apolipoprotein E |
| APP | Amyloid Precursor Protein |
| BDNF | Brain-Derived Neurotrophic Factor |
| ChAT | Choline Acetyltransferase |
| CSF | Cerebrospinal Fluid |
| DSST | Digit Symbol Substitution Test |
| EOAD | Early-onset Alzheimer's Disease |
| ELISA | Enzyme-linked Immunosorbent Assay |
| FDA | Food and Drug Administration |
| FFX | Fixed Effects Analysis |
| fMRI | Functional Magnetic Resonance Image |
| FTD | Frontotemporal Dementia |
| HWE | Hardy-Weinberg Equilibrium |
| IHCC | Immunocytochemistry |
| IOG | Inferior Occipital Gyrus |
| ISH | In Situ Hybridization |
| ITG | Inferior Temporal Gyrus |
| LBD | Lewy Body dementia |
| LiG | Lingual Gyrus |
| LOAD | Late-onset Alzheimer's Disease |
| mBDNF | mature Brain-Derived Neurotrophic Factor |
| mdMCI | multiple domain Mild Cognitive Impairment |
| MCI | Mild Cognitive Impairment |
| MFG | Middle Frontal Gyrus |

| | |
|---|---|
| MMSE | Mini-Mental State Examination |
| MoCA | Montreal Cognitive Assessment |
| mRNA | messenger Ribonucleic Acid |
| naMCI | non-amnestic Mild Cognitive Impairment |
| NCI | No Cognitive Impairment |
| NFTs | Neurofibrillary Tangles |
| NMDA | Glutamate N-methyl-D-aspartate |
| NOS | Nitric Oxide Synthase |
| NOV | Number of Activated Voxels |
| OfUG | Occipital Fusiform Gyrus |
| PD | Parkinson Disease |
| PCu | precuneus |
| PCR | Polymerase Chain Reaction |
| PET | Positron Emission Tomography |
| PoG | Postcentral Gyrus |
| PrG | Precentral Gyrus |
| PSEN-1 | Presenilin-1 |
| PSEN-2 | Presenilin-2 |
| p75NTR | p75 Neurotrophin Receptor |
| RT-PCR | Reverse Transcriptase - Polymerase Chain Reaction |
| sdMCI | single domain Mild Cognitive Impairment |
| SFG | Superior Frontal Gyrus |
| SMC | Supplementary Motor Cortex |
| SNP | Single Nucleotide Polymorphism |
| SOG | Superior Occipital Gyrus |
| TrkB | Tyrosine receptor kinase B B |
| TMT B | Trail Making Test b |
| USM | Universiti Sains Malaysia |
| VAChT | Vesicular Acetylcholine Transporter |
| VAD | Vascular Dementia |
| WBB | Wii Balance Board |
| 7-NI | 7-Nitroimidazole |

## LIST OF APPENDICES

**PENGENALPASTIAN POLIMORFISME GEN FAKTOR NEUROTROFIK BERASAL *(BDNF) VAL66MET* SEBAGAI PENANDA BIO KEPADA PRESTASI KOGNITIF OTAK YANG SEMAKIN TUA HASIL TINDAKBALAS TERHADAP 8 MINGGU LATIHAN EXERGAMES**

## ABSTRAK

Polimorfisme gen *BDNF Val66Met* adalah faktor yang dipercayai merubah prestasi kognitif bagi penuaan otak dengan pembawa *Met* menunjukkan kemerosotan ketara dalam kebolehan kognitif. Oleh itu, Kajian 1 mengkaji taburan polimorfisme gen *BDNF Val66Met* dalam populasi penuaan dan perkaitannya dengan prestasi kognitif. 206 orang tua berumur 55 – 80 tahun terlibat dalam kajian ini dan dikategorikan berdasarkan skor *Mini Mental State Examination* (MMSE): kumpulan Kawalan (MMSE > 26; n = 103) dan Kes (MMSE ≤ 26; n = 103). Penemuan menunjukkan bahawa taburan polimorfisme gen *BDNF Val66Met* adalah berbeza secara ketara antara kumpulan yang dikaji. Sebaliknya, genotip *BDNF* mempengaruhi kemerosotan kognitif sebanyak dua kali ganda. Oleh itu, Kajian 2 dijalankan untuk mengkaji kesan polimorfisme gen *BDNF Val66Met* terhadap prestasi kognitif penuaan otak sebagai tindak balas kepada latihan Exergames selama 8 minggu. 42 peserta daripada kumpulan Kes dalam Kajian 1 telah dibahagikan kepada 3 kumpulan dan melakukan senaman selama 30 minit, 3 hari setiap minggu untuk 8 minggu: Exergames (senaman menggunakan Ring Fit Adventure Nintendo Switch; n = 14), kumpulan Konvensional (latihan senaman yang sama tanpa menggunakan exergames; n =14) dan Kawalan (tiada latihan senaman; n = 14). Hasil kajian menunjukkan bahawa terdapat perbezaan secara signifikan bagi skor ujian neuropsikologi antara kumpulan yang dikaji. Analisis fMRI menunjukkan bahawa lebih banyak bahagian

otak dengan pengaktifan yang lebih tinggi daalam kumpulan Exergames berbanding dengan kumpulan Konvensional. Bagi genotip *BDNF*, ukuran berulang ANOVA mendapati bahawa prestasi kognitif adalah lebih tinggi bagi genotip *ValVal* untuk skor MoCA dan menunjukkan peningkatan isipadu hipokampus selepas senaman Exergames. Alel *Met* juga dikaitkan dengan peningkatan skor ujian neuropsikologi bagi kedua-dua mod latihan senaman. Walaupun senaman Exergames tidak menunjukkan peningkatan keseluruhan, terdapat trend yang menarik dilihat bagi genotip *ValVal*. Ini membuktikan bahawa latihan Exergames merupakan pilihan latihan fizikal-kognitif yang menarik dan menjanjikan kesan yang positif di kalangan bagi populasi yang semakin tua.

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## ABSTRACT

The *BDNF Val66Met* gene polymorphism is a plausible factor for the changes in cognitive performance of the aging brain with *Met* carriers showed a greater impairment in cognitive ability. Therefore, study 1 examined the distribution of *BDNF Val66Met* gene polymorphism in the aging population and its association with cognitive performance. 206 older adults aged 55 – 80 years were enrolled in the study and divided based on their Mini Mental State Examination (MMSE) score: control group (MMSE $> 26$; n = 103) and case group (MMSE $\leq 26$; n = 103). The results showed that the distribution of *BDNF Val66Met* gene polymorphism was significantly different between the studied groups. Conversely, the *BDNF* genotype was associated with a twofold risk of cognitive impairment. Therefore, Study 2 was conducted to examine the effect of the *BDNF Val66Met* gene polymorphism on cognitive performance in the aging brain in response to 8 weeks of exergames training. 42 participants from the case group in study 1 were subjected to 3 groups with different training methods for 30 minutes, 3 days per week for 8 weeks: exergames (training with Ring Fit Adventure for Nintendo Switch; n = 14), conventional training (similar training without exergames; n = 14), and control group (no training; n = 14). The results showed that neuropsychological test scores had significant differences between the studied groups. fMRI analysis showed that more brain areas with greater activation were detected in the exergames group as compared to the conventional group. With

respect to *BDNF* genotype, a repeated measure of ANOVA revealed that cognitive performance was better in the *ValVal* genotypes on MoCA scores, and hippocampal volume tended to improve after the exergames intervention. In addition, the *Met* allele was associated with an improvement in neuropsychological test scores in response to both types of exercise training. Although the intervention did not produce overall improvements, there were interesting trends in the data for the *ValVal* genotype after exergames training. This demonstrates that exergames training appears to be an appealing and promising means of concurrent physical-cognitive training in the aging population.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background and Scope of the Research

Despite advances in treating degenerative diseases associated with ageing, dementia remains one of the most critical global health problems (Nemeth *et al.*, 2017). Forecasts indicated that 74.7 million individuals around the world will be living with dementia by the year 2030 (Irazoki *et al.*, 2020). The condition known as mild cognitive impairment (MCI), a transitional stage between normal aging and dementia, is also prevalent in older adults (Petersen *et al.*, 2001). MCI affects 10 % to 20 % of those over 65 years of age (Petersen, 2011). MCI can affect a single cognitive domain (sdMCI) or many domains (mdMCI), and it can be either amnesic (aMCI) or non-amnesic (naMCI) (Petersen *et al.*, 2014). aMCI was categorized based on the poor results of the neuropsychological tests assessing episodic memory performance, meanwhile impairments in cognitive areas other than memory such as language, executive functioning or visuospatial ability indicated naMCI (Petersen *et al.*, 2014). aMCI has often been attributed to Alzheimer's disease (AD) (Lange *et al.*, 2018), contributing to 50 % to 60 % of dementia cases. In contrast, naMCI may increase the risk of developing other dementias, such as dementia with Lewy bodies and frontotemporal dementia (Ferman *et al.*, 2013).

Because there is currently no treatment that can reverse the effects of dementia, researchers have focused on finding ways to slow the course of cognitive decline and delay the onset of symptoms as long as possible. Cognitive reserve and neuroplasticity have become possible causes for minimising cognitive decline (Soldan *et al.*, 2017). Multiple factors, including educational level, heredity, profession, socioeconomic status, physical well-being, diet and lifestyle, may influence brain cognitive capacity

and plasticity throughout the course of a lifetime (Sampedro-Piquero & Begega, 2017). Thus, identifying characteristics that affect cognitive function in AD and dementia requires a knowledge of the root mechanisms of the disease. Neurotrophins such as brain-derived neurotrophic factor (BDNF) have been studied for their potential role in age-related cognitive impairment (Nordvall, Forsell & Sandin, 2022; Numakawa & Odaka, 2022). Thus, Mattson *et al.* (2004) noted that the decreased of BDNF signalling may have aided in the progression of several of age-related illness. Additionally, the main molecular focus for the discovery of medications to alleviate neurological disorders is BDNF (Binder & Scharfman, 2004). Therefore, verifying BDNF as the primary factor controlling neurotrophin segregation, neuronal survival, and synaptic plasticity is crucial (Binder & Scharfman, 2004). Given that BDNF is essential for the survival and normal functioning of neurons, both of which are compromised in individual with AD, it stands to reason that BDNF has a substantial influence in the mental abilities of those diagnosed with the disease.

Neurodegeneration was formerly considered to be caused by variations in BDNF expression and signalling (Ventriglia *et al.*, 2013). The result is consistent with the findings by Balaratnasingam and Janca (2012), who found modifications in levels of BDNF in the brains of patients with AD after death. Alterations in BDNF functionality have also been found in various neurodegenerative diseases. As a result, low BDNF levels have been linked not just to Alzheimer's disease (Zuccato & Cattaneo, 2009) but also to several mental health problems and neurological diseases (Diniz & Teixeira, 2011). Among the different genetic factors associated with BDNF, the most studied polymorphism related to cognitive performance in neurodegenerative diseases is the *BDNF Val66Met* gene polymorphism (Bessi *et al.*, 2020; Brown *et al.*, 2020; Cechova *et al.*, 2020; Abanmy *et al.*, 2021). This polymorphism occurs due to

single-nucleotide polymorphism (SNP) in the *BDNF* gene that results in a switch from valine (*Val*) to methionine (*Met*) at codon 66 (Shen *et al.*, 2018). The structure of the pro-domain of the gene is altered in people with *Met* variant of the *BDNF* gene, referred to as *BDNF ValMet* heterozygotes and *MetMet* homozygotes (Shen *et al.*, 2018). In addition, carriers of two copies of the valine allele (*Val*) have been reported to have higher BDNF activity than individuals with the methionine allele (*Met*) (Dincheva, Glatt & Lee, 2012). This polymorphism has been associated with variations in episodic memory performance, with an individual with the *Met* allele performing worse than individuals with both *Val* alleles (Dincheva, Glatt & Lee, 2012).

One of the most powerful non-pharmacological strategies for improving cognitive function in the elderly is regular physical activity (Gomes-Osman *et al.*, 2017). The increased release of BDNF immediately following exercise is one of the best-known processes underlying the cognitive benefits triggered by exercise (Hamilton & Rhodes, 2015). However, possession of a *Val* or *Met* allele could also have an effect on the variability of individual's reaction to exercise programs. Although there is overwhelming empirical findings that exercise improves cognitive performance (Mandolesi *et al.*, 2018), significant intra- and inter-subject differences in the cognitive effects of exercise have been found between *ValVal* and *ValMet* subjects (Erickson *et al.*, 2013; Nascimento *et al.*, 2015; Thibeau *et al.*, 2016; Canivet *et al.*, 2017; Watts, Andrews & Anstey, 2018). In addition, engaging older adults in physical activity or exercise programs is often difficult. The most significant barriers are behavioural characteristics such as restlessness and apathy (Kolanowski, Litaker & Buettner, 2005), disorientation, and decreased interest (Santen *et al.*, 2018). Technological advances have created new opportunities to engage older adults,

particularly those with cognitive impairments, in exciting ways through using exergames (Dove & Astell, 2017). Exergames refer to a kind of video games that necessitate participants to engage in physical motions in order to actively participate and successfully perform tasks prompted by visual cues (Amjad *et al.*, 2019). Various terms such as active gaming and motion-based virtual reality gaming are employed to describe the games. These games are used extensively as a form of recreation however, there has been growing interest in recent years in the potential benefits of exergames among aging population with MCI or dementia (Dietlein *et al.*, 2018; Santen *et al.*, 2018; Swinnen *et al.*, 2020). Exergames require players to perform bodily movements in response to auditory, somatosensory, and visual stimuli that combine physical exercise and mental functions (Meekes & Stanmore, 2017; Kappen, Mirza-Babaei & Nacke, 2018). According to a recent qualitative study, elders living in long-term care homes reported positive experiences with exergaming (Swinnen *et al.*, 2020). Mean participation in all 24 exergames sessions occurred three times per week over an 8-week was 79.3 % (Swinnen *et al.*, 2021). In a systematic review by Zhao *et al.* (2020) on the effectiveness of exergames in improving cognitive and physical functioning, most studies found the duration of exergames programs to be between 6 and 8 weeks, with eight weeks being the median (range of 4-24 weeks) (Padala *et al.*, 2017; Mrakic-Sposta *et al.*, 2018; Karssemeijer *et al.*, 2019). This finding indicated that a duration of between 6 and 8 weeks is sufficient to report on exergames' effectiveness, although the outcome may vary due to the variety of exergame interventions used (Zhao *et al.*, 2020).

Despite the positive findings on the effects of *BDNF* genotype on response to conventional training, to our knowledge, no research has investigated the impact of exergames on cognitive performance in the aging population with different *BDNF*

genotypes. This study is essential to determine whether the *BDNF Val66Met* gene polymorphism impacts cognitive performance in response to exergames training. This investigation could assist in detecting the people who would reap the most benefit from participating in this training program. Thus, the objective of this study was to investigate whether the *BDNF* genotype of the elderly population influences their training responses following an 8-week exergames training program. This study hypothesizes that the cognitive performance of the aging population after the exergames training program depends on the *BDNF* genotype. It is expected that the cognitive performance induced by the exergames program will be significant in individuals with the *Val* allele than in individuals with the *Met* allele. The desired outcomes from this study could provide as a basic guideline for clinicians or exercise physiologists to optimize their approach to a specific training program that can improve the cognitive performance of their patients.

## 1.2 Statement of Problems

Despite the widespread and well-known advantages of physical activity on cognitive performance, the likelihood of individuals achieving adequate levels of physical activity diminishes as they age. Older individuals may perceive health recommendation regarding exercise as targeting younger individuals, or they may believe that their physical constraints hinder their ability to engage in physical exercise (André & Agbangla, 2020). Moreover, Collins *et al.* (2022) have documented that around 66 % of older adults discontinued their involvement prior to the commencement of the exercise intervention. Nevertheless, it is widely acknowledged that the commitment to health-prevention intervention plays a crucial role in attaining favourable results in physical activity, leading to an improved quality of life (André &

Agbangla, 2020). A study showed that an exergames training program has the ability to encourage long-term adherence in AD because it is simple to implement and does not demand greater training intensity and minimal training time (Suzuki *et al.*, 2012). It is suggested that improvement in cognitive function could be achieved through exergames, as they are physically demanding and require concentration and cognitive effort (Netz, 2019). A study by Lee (2016) found that patients with cognitive decline benefited from a virtual reality exercise program using Nintendo Wii consoles over the course of 12 weeks.

Currently, the mechanism by which exergames improve cognitive function in aging population with MCI or dementia is unclear due to heterogeneity among studies in terms of the study characteristics, including differences in the duration, frequency range, and types of games employed (Cai *et al.*, 2023). One study suggests that exercise training increases BDNF levels in the brain, which is a member of the neurotrophic family and is responsible for the formation of new brain cells in the hippocampus, helping neurons remain vital, strengthening synapses (nerve-to-nerve connections) and promoting learning and memory (Sleiman *et al.*, 2016). Research indicates that low BDNF expression is a critical factor in the development of AD (Sleiman *et al.*, 2016), as lower BDNF levels are correlated with cognitive impairment (Sleiman *et al.*, 2016). According to Liu *et al.* (2009), moderate treadmill training and cycling upregulated BDNF signalling in the hippocampus, with both training protocols having different effects on different brain regions and their functions. A study by Anderson-Hanley *et al.* (2012) comparing exergames-based training interventions to traditional training interventions in older adults found that the exergames group experienced a more significant increase in BDNF than those trained with conventional

cycling. Based on these findings, the influence of exercise on cognitive performance in the aging population with MCI or dementia may depend on the type of exercise.

Previous studies also suggest that genetic variations contribute to interindividual variability in *BDNF* gene expression and have influenced cognitive performance and motor learning in healthy adults in response to exercise (Liu *et al.*, 2009; Anderson-Hanley *et al.*, 2012). *BDNF* gene expression is regulated by the *BDNF Val66Met* gene polymorphism (Song, Yu & Tan, 2015). Of the two alleles of the *BDNF Val66Met* gene, the carriers of two copies of the *Val* allele have been shown to have elevated BDNF activity than individuals with the *Met* allele (Buchman *et al.*, 2016). The relevance of the *BDNF Val66Met* gene polymorphism to cognitive performance was corroborated in a recent study by Azeredo *et al.* (2017) in 87 subjects over 55 years of age, which found that carriers of the *BDNF Met* allele had lower delayed verbal recall scores and memory performance compared with older adults with the *Val* allele. With the findings of Azeredo *et al.* (2017) and similar findings from previous study (Buchman *et al.*, 2016), the mediating effect of BDNF on cognitive function may vary and depend on the *BDNF* genotype.

In light of the above findings, genetic differences in BDNF levels and type of exercise could affect individual response to exercise and thus influence the body's ability to reap the benefits of a particular intervention. To our knowledge, no study has investigated the effect of exergames on cognitive performance in the aging population with different *BDNF Val66Met* genotypes. In addition, there is a limited study in Malaysia on the association between *BDNF Val66Met* gene polymorphism and the cognitive performance of the aging population. The study of the distribution pattern of *BDNF Val66Met* in the aging population of Malaysia is essential to confirm the association between *BDNF Val66Met* and susceptibility to AD or dementia in

Malaysia. The association data will be a helpful guideline or baseline to determine whether exergames' effects on the ageing population's cognitive performance differ according to *BDNF* genotype, that would assist in identifying people who might benefit more from this exercise routine.

## 1.3 Conceptual Framework

Figure 1.1 shows the conceptual framework around which this study is built on. The model assumes that an exergames training program can improve cognitive performance in the aging population and that the level/score of cognitive performance in response to an 8-week training program will differ between carriers of the *BDNF Val66Met* genotype. More specifically, it is hypothesized that individuals with the *ValVal* genotype would achieve more significant improvements in the selected variables than individuals with the *ValMet* and *MetMet* genotypes.

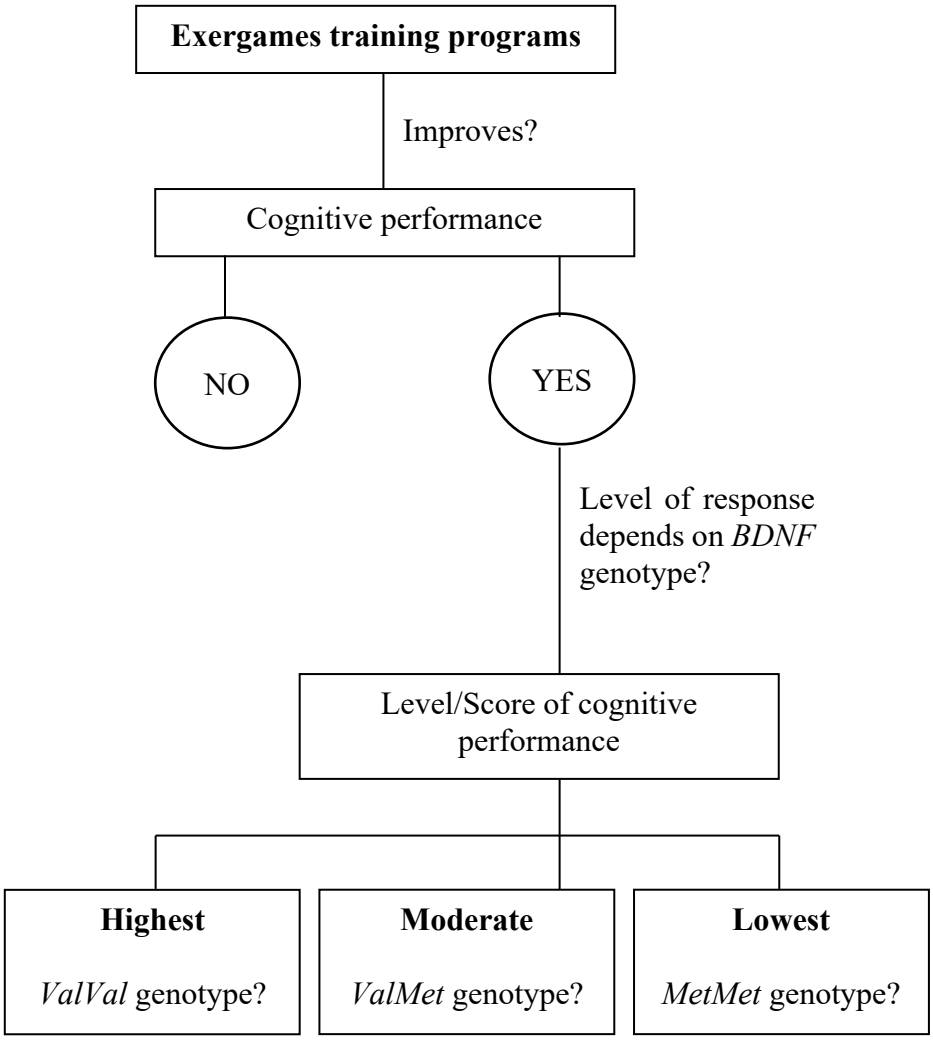


Figure 1.1 Conceptual model of the exergames training program and *BNDF Val66Met* genotype.

## 1.4 Objectives of the Research

The primary aim of this study was to establish whether the *BDNF Val66Met* gene polymorphism can serve as a biomarker for cognitive performance in the aging brain, in response to an 8-week exergames training program. The specific objectives of this research were as follows:

i. To determine the distribution of the *BDNF Val66Met* gene polymorphism in the aging population from Hospital Seberang Jaya and Klinik Kesihatan Kepala Batas by comparing the data in the general population, ethnicity, gender, and their association with cognitive performance.

ii. To investigate the effect of *BDNF Val66Met* gene polymorphism on cognitive performance as measured by neuropsychological test, peripheral BDNF levels, fMRI Stroop task for brain activation, and hippocampal volume in MCI individuals in response to 8 weeks of exergames training.

iii. To compare the effects of exergames and conventional training programs on cognitive performance, peripheral BDNF level, brain activation, and hippocampal volume of MCI individuals.

## 1.5 Significance of the Research

The knowledge gained from the series of experiments in this PhD project provides a better understanding of the role of the genetic component in the cognitive performance of the aging human brain through various forms of physical activity. In addition, this research provides more information about the *BDNF Val66Met* gene polymorphism and exercise adaptation. In order to determine the effects of *BDNF Val66Met* gene polymorphism on the cognitive performance of the aging brain in response to an exergames intervention, this study will benefit the community, especially patients and caregivers, in managing dementia patients based on their genetic characteristics. Moreover, the results of this PhD project will also yield important data that can directly benefit patients and indirectly improve their quality of life, with each session of the training program designed as a rehabilitation-like treatment with positive feedback and reinforcement of the patient's performance.

## 1.6 Operational Definition

The list of operational definitions is shown in the Table 1.1.

Table 1.1 Operational definition

| Abbreviation | Operational Definition |
|---|---|
| Brain-Derived Neurotrophic Factor (BDNF) | BDNF is a protein that is essential for stimulating neurons' survival, differentiation and development in the central nervous system. It has been associated to several physiological functions such as synaptic plasticity, learning, and memory. Methods such as enzyme-linked immunosorbent assay (ELISA) and Western blotting can be applied to determine the concentration of BDNF that contained within a biological sample including blood or brain tissue. |
| Mild Cognitive Impairment (MCI) | MCI is often regarded an intermediate phase between normal aging and dementia. It is a neurological condition marked by memory difficulties and evidence of a slight deterioration in cognitive performance, but does not affect daily functioning which is not acute enough to fulfil dementia's criteria. |
| Alzheimer's Disease (AD) | AD refers to a progressive and degenerative illness of the brain that affects multiple factors of cognitive performance such as memory, thinking, and behaviour. Amyloid plaques and neurofibrillary tangles, resulting in the brain cells death and loss of synaptic transmission are the hallmarks of AD. |
| Dementia | Dementia is characterized by a deterioration in cognitive ability that impairs with daily activities. Dementia is a basic term that encompasses a range of conditions that cause changes in memory, thinking, behaviour, and the ability to perform everyday activities. The specific symptoms and the rate at which they progress depends on the person and the type of dementia. |
| Exergames | Exergames is used to describe video games that encourage active play by using motion-sensing technology like a Kinect or Wii remote to track the player's motions and react accordingly in real time. Exergames aim to make exercise and other forms of physical activity more appealing by introducing gameplay features like competitiveness, achievement, and feedback into their design. |
| Physical exercise | Physical exercise refers to any physiological activity performed by skeletal muscles that enhance energy consumption beyond normal resting state which comprises of variety of tasks, such as cardio workouts like jogging, cycling, or swimming, strength training like lifting weights, and flexibility and balance exercises including tai chi and yoga. |

Table 1.1 Continued

| | |
|---|---|
| Cognitive performance | Cognitive performance is the capacity of an individual to accomplish activities associated with different cognitive domains such as language, memory, attention, processing speed and executive function. A range of approaches, comprising neuropsychological tests, performance-based activities, and self-report assessments, can be used to assess cognitive performance. |

## 1.7 Thesis Structure

This work includes two studies addressing the role of certain genetic variants indicated by the *BDNF Val66Met* gene polymorphism as a biomarker of cognitive performance in the aging brain in response to 8 weeks of exergames training. A comprehensive overview of the topic is covered in the Chapter 2 of the literature review. The first study, presented in Chapter 3, aimed to examine the distribution of *BDNF Val66Met* gene polymorphism in the aging population of Malaysia and its association with cognitive performance. The investigation was then extended in the second study examining the effects of *BDNF Val66Met* gene polymorphism on cognitive performance in the aging brain in response to 8 weeks of exergames training in Chapter 4. Finally, Chapter 5 presents the overall conclusion of this PhD project.

## 1.8 Research Overview

*BDNF* gene is the most studied gene associated with human cognitive performance (Girotra *et al.*, 2022). Therefore, this thesis aimed to present the results of two studies that corroborated the role of *BDNF Val66Met* gene polymorphism on the cognitive performance of the aging brain within the Malaysian population.

A case-control study was carried out in the first study to determine if the distribution of *BDNF Val66Met* gene polymorphism differed in the aging population and their association with poor cognitive performance to be able to serve as a standard

reference before establishing the effects of this polymorphism on cognitive performance of the aging brain in Malaysian population. Therefore, 206 of the elderly population (89 males, 117 females), (175 Malays (85 %), and 31 Others (Chinese, Indian, and Other Bumiputras (15 %)), mean age 68.27 ± 6.9 years were selected as participants in the first study. Based on their MMSE score, 103 participants with an MMSE score of ≤ 26 were assigned to the Case group (35 males, 68 females) mean age 70.21 ± 7.6 years, while another 103 participants with an MMSE score of > 26 were classified as Control group (54 males, 49 females) mean age 66.33 ± 5.6 years. Each participant retrieved a DNA sample via buccal cell, and the *BNDF* genotype was then identified through Polymerase Chain Reaction (PCR).

The effects of *BDNF Val66Met* gene polymorphism on the cognitive performance of the aging brain in response to an 8-week of exergames training was examined in the second study. A total of 42 participants (14 males, 28 females) mean age 72 ± 7.6 years from the Case group in the first study were involved in the second study. The participants were then assigned to two different exercise methods (Exergames and Conventional groups) with one Control group. They performed exercise training three days per week for eight weeks. The Exergames group completed the exercise training using the Ring Fit Adventure for Nintendo Switch. In contrast, the Conventional group performed a similar exercise program as the Exergames group but without using the exergames and was assisted by the exercise trainer. Participants had completed several assessments before (pre-test) and after (post-test) to exercise training which comprised of blood samples collection for measuring BDNF serum level and *BDNF* gene expression, neuropsychological tests which consisted of MoCA, Trail Making Test B (TMT B) and Digit Symbol

Substitution Test (DSST), and functional magnetic resonance imaging (fMRI) for brain activation analysis and hippocampal volume measurements.

Figure 1.2 shows the flowchart for the entire research process. The next chapters go into the great detail about the extensive methodologies that were used in each study. The Human Research Ethics Committee approved the study protocols in Universiti Sains Malaysia (Appendix A) and the Medical Research and Ethics Committee in Kementerian Kesihatan Malaysia (Appendix B).

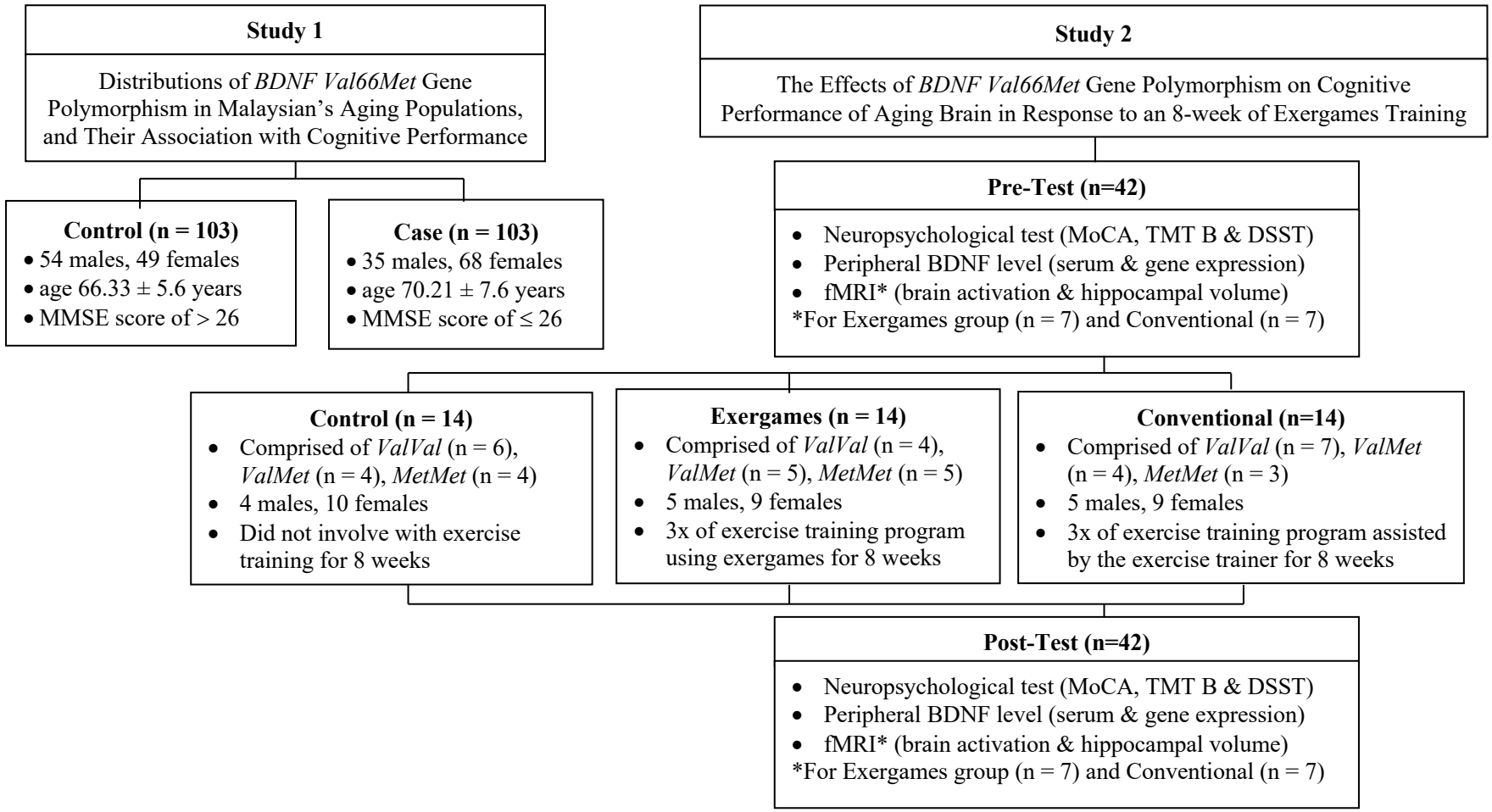


Figure 1.2 The general research processs

*Abbreviations:* Montreal Cognitive Assessment (MoCA), Trail Making Test B (TMT B), Digit Symbol Substitution Test (DSST), Functional magnetic resonance imaging (fMRI)

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

The brain experiences oxidative stress in several ways during normal aging. One of the symptoms of aging is memory loss (Rekatsina *et al.*, 2020). Mild cognitive impairment (MCI) is typically used to describe significant memory loss without loss of other cognitive abilities (Tangalos & Petersen, 2018). MCI is the stage that occurs between the typical aging-related cognitive decline and the more severe dementia-related decline. According to various studies, people with MCI are more susceptible to dementia, particularly AD, where individuals with MCI have a three- to fivefold increased risk of developing dementia compared to their peers (Eshkoor *et al.*, 2015; Jia *et al.*, 2020; Landeiro *et al.*, 2020; Alvi *et al.*, 2022). Reducing the progression of MCI in its initial phases may be advantageous, as this could enhance cognitive function and lower the likelihood of dementia. Thus, the goal of this chapter is to present an overview of relevant research on normal aging, MCI, and dementia, including current assessment and treatment, using the existing literature as a basis for answering the present research questions.

### 2.1.1 Aging

Throughout human history, aging, which was defined generally as the time-dependent functional decline which affects most organisms, aroused curiosity and stimulated the imagination of many people (López-Otín *et al.*, 2013). Nevertheless, it has only been 40 years since the discovery of the first long-lived variants of *Caenorhabditis elegans*, bringing a new era to aging research (Klass, 1983). Aging is described as a progressive decline in functional ability at the molecular, cellular, tissue, and organismal levels, which causes the incidence of degenerative diseases in

mammals (He *et al.*, 2022). Inevitably, the aging process causes an organism to become frailer, more susceptible to illness, and more likely to die. Aging is the main significant predictor for many human disorders, notably neurodegeneration, diabetes, cardiovascular disease, cancer, and osteoporosis (Booth & Brunet, 2016).

According to Harman's Theory of Aging, "Aging is characterized as the progressive accumulation of changes over time that is connected with or responsible for the increased susceptibility to disease and death that comes with advancing age" and "the sum of the deleterious free radical reactions that constantly occur throughout the cells and tissues constitutes the process of aging or is a main contributor to it" (Harman, 1956). Historically, free radical and oxidative reactions were hypothesized to disrupt biological mechanisms and speed up aging (Harman, 1956). Although most scientists believe that oxidative stress and free radicals serve a minimal role in aging, it is hard to dispute the widely established notion that aging is due to multiple causes that degrade the structures and functions of an organism's cells, molecules, and organs (Ermolaeva *et al.*, 2018; Alberti & Hyman, 2021). The aging factors included oxidative stress, side reactions, glycation, telomere shortening, mutations, and protein aggregation. Despite its origin, molecular damage is still the proximate cause underlying the process of aging (Zimniak, 2012). Even though hyperfunction is the primary cause of aging, it kills organisms not through catastrophic relapses but by inducing molecular damage (Zimniak, 2012).

The accumulation of molecular damage caused by abnormalities in the molecular machinery of life has long been thought to be crucial to the aging process (Gladyshev, 2013). The intrinsic imperfections of biological functioning give rise to the occurrence of damage through all biological processes, spanning from the molecular to cellular levels. The extent of the damage is extensive and cannot be

remedied completely, as it is largely imperceptible to the process of natural selection and expresses itself in the form of aging. To a considerable extent, the cumulative damage is not random as it is inadvertently inscribed in the genome because each biomolecule generates specific forms of damage (Gladyshev, 2013). However, it is unclear how this damage is caused, if it is intentional, why it cannot be eradicated from cells, and whether it is stochastic. In addition, it is unknown whether the damage is the cause of aging or merely a by-product of the process. Although the idea that cumulative damage promotes aging (Ogrodnik, Salmonowicz & Gladyshev, 2019) is widely accepted, nearly every aspect of this theory is controversial, with many researchers denying the idea as a significant contributor (McHugh & Gil, 2018; Ferrucci *et al.*, 2020; Lemoine, 2020). In other words, what we perceive and identify as aging is the increasing degradation of these structures and functions. This damage contributes to the formation of pathological disorders, which eventually lead to mortality based on the concept referred to as the general theory of aging (Zimniak, 2012) or the standard theory of aging (Liochev, 2015).

In addition, the development of healthcare facilities has substantially increased the expected lifespan, resulting in a substantial rise in the proportion of people aged 65 and older (Eshkoor *et al.*, 2015). Dementia and AD can be diagnosed as part of memory loss in the elderly, a common side effect of aging characterized by impairments in episodic and recent memory (Fabrício, Chagas & Diniz, 2020). Factors such as age, gender, educational status, and depressive diagnosis may play a role in determining why memory impairment rates vary between populations (Kim *et al.*, 2017). It is anticipated that the global elderly population will increase by 21 % over the next five decades, with a 140 % increase in the elderly population in growing countries and a 51 % increase in developed countries (Eshkoor *et al.*, 2015). Given the

rapid demographic aging in low and middle-income societies, it is crucial to initially recognize those at risk of acquiring dementia to focus on preventive measures. Since MCI is a phase that occurs between normal aging and cognitive decline that is associated with dementia, thus, the detection of MCI can provide an essential impact on the early diagnosis, prevention, and effective medication for treating the disease (Untari *et al.*, 2019).

### 2.1.2 Mild Cognitive Impairment (MCI)

MCI is a state that causes cognitive impairment and difficulty carrying out fundamental daily tasks and was hypothesized as a precursor to dementia (Tangalos & Petersen, 2018). It is a phase that occurs between healthy aging and dementia, and it most usually manifests itself as AD (Berkes *et al.*, 2020). Even though AD is the most prevalent cause of MCI, other neurodegenerative, systemic, and psychiatric disorder can also cause cognitive impairment before AD manifests itself (Huey *et al.*, 2013). Approximately 6.7 % up to 25.2 % of adults over 60 have MCI. It affects more women than men and is worse as people get older and educated less (Chang *et al.*, 2017; Petersen *et al.*, 2018). Nevertheless, the effects are inconsistent since most research employs varying definitions of MCI. Memory loss was once the primary diagnostic criterion for MCI. Still, a more inclusive description is currently being used, which includes impairment in single or numerous non-amnestic cognitive domains, with or without memory loss (Jongsiriyanyong & Limpawattana, 2018). According to Peterson *et al.* (2018), aMCI is a diagnosis in which memory dysfunction predominates whole in non-amnestic MCI, and there is impairment in other cognitive aspects more apparent such as language, visuospatial and executive function.

The clinical features of MCI include concern over a decrease in cognition, cognitive dysfunction in one or more domains, impairment in regular daily activity,

and the absence of dementia (Arevalo-Rodriguez *et al.*, 2021; Bradfield, 2021). Detailed questioning of the patient's history with qualified informants is essential to detect the clinical clues. Implementing adequate cognitive screening tests is another critical component of the clinical evaluation of MCI patients. One of the instruments that have been used to screen cognitive is the Montreal Cognitive Assessment (MoCA), with a cut-off value of 24/25 (Pinto *et al.*, 2019) and the test's sensitivity and specificity for the aging population was reported to be 80.48 % and 81.19 %, respectively (Ciesielska *et al.*, 2016). At the cut-off of 26 (Gils *et al.*, 2022), it demonstrated a sensitivity between 80 and 100 % and a specificity between 50 and 76 % (Langa & Levine, 2014). However, the MoCA results may also be influenced by other factors such as the educational level, lifestyle circumstances and ethnic diversity (O'Driscoll & Shaikh, 2017; Miyakawa-Liu *et al.*, 2022). The Cantonese Chinese adaptation with a cut-off score of 22/23 revealed a sensitivity of 78 % and specificity of 73 % in detecting the amnestic form of MCI (Chu *et al.*, 2015). Furthermore, after controlling for variables such as education level, the ideal cut-off for the education-adjusted MoCA score was determined to be 25, corresponding to a sensitivity of 61 % and a specificity of 97 % (Gagnon *et al.*, 2013).

Diverse clinical presentations of MCI are expected due to the various causes of the condition, which include systemic diseases, neurodegenerative disorders, and psychiatric problems (Siafarikas *et al.*, 2018; Lau *et al.*, 2021). Few outcomes of MCI are explicable by its pathogenesis, such as reversion to normal aging, stability, or progression to dementia (Petersen, 2016). Koepsell and Monsell (2012) reported in a study including 3,020 persons diagnosed with MCI that roughly 16 percent of participants with MCI returned to normal or near-normal cognition after approximately one year. However, studies showed that the probability of switching to

MCI or dementia over the next three years was considerably higher among those with a history of MCI who recovered than those without a history of MCI (Uddin *et al.*, 2019; Arevalo-Rodriguez *et al.*, 2021). Although some cognitive decline is expected with advancing age, there is mounting evidence that some forms of cognitive impairment can serve as a precursor to dementia (Fiorini, Luzzi & Vignini, 2020; Moura *et al.*, 2022; Tran *et al.*, 2022). MCI is a diverse condition, and there is still debate regarding several construct elements. However, this paradigm's value lies in the knowledge that dementia is not a dichotomous condition and that by improving our knowledge of the layers of transition, we can better understand the cognitive decline and eventually benefit patients. Choosing which therapies, whether medical or non-medical, can satisfy our patients' needs when a correct diagnosis has been made (Tangalos & Petersen, 2018).

Recently, studies have been reported on MCI biomarkers such as BDNF (Mori *et al.*, 2021; Ng *et al.*, 2021; Huang *et al.*, 2021). It is hypothesized that a lower level of BDNF is associated with the progression of neurodegenerative disorders, notably MCI (Al-Rawaf, Alghadir & Gabr, 2021). BDNF levels were considerably lower in individuals with cognitive impairment compared to healthy controls (Ng *et al.*, 2019). Moreover, increased BDNF serum levels were related to improved cognitive function in healthy-aged individuals (Mora *et al.*, 2019). Therefore, it is also essential to examine the association between BDNF and MCI with various variables of cognitive function in the aging population, as it can be a potential treatment strategy for lowering the risk of neurodegenerative disorders, especially MCI, in the future.

## 2.2 Dementia

### 2.2.1 Current Literature

According to the American Psychiatric Association (2013), dementia is described as a severe neurocognitive disease with a decline of intellectual abilities (medically known as cognitive function) severe enough to impair social or occupational functioning. Dementia is characterized by a reduced in global cognitive and executive functioning that can causes a decrease in personal and functional abilities and a reduction in mobility and independence (Purser & Lonie, 2019; Malik *et al.*, 2022). There are different types of dementia, some of which are reversible and some of which are not the most common forms are AD which accounts for about half to seventy percent of dementia cases, and vascular dementia, which accounts for about eighteen to twenty percent of dementia cases (Lee *et al.*, 2021; Hermann & Zerr, 2022; Malik *et al.*, 2022). Beginning around the age of 65, the chance of having dementia increases nearly every five years (Rone-Adams *et al.*, 2013). The occurrence of dementia is predicted to increase dramatically over the next century due to the aging of the population and the subsequent rise in the average lifespan. This number is projected to double by 2030 and quadruple by 2050 (Rone-Adams *et al.*, 2013).

Aging is a major risk factor for all types of dementia. For example, AD affects between 5 and 10 % of those aged 65 and above and half of those aged 85 and above (Arvanitakis, Shah & Bennett, 2019). Ethnicity, gender differences and genetic factors including the apolipoprotein E (ApoE) and *BDNF* gene are non-modifiable risk factors for AD (Head *et al.*, 2016; Terracciano *et al.*, 2017). Hypertension, diet intake, diabetes, and physical and social inactivity are modifiable risk factors for dementia due to any cause (Gupta *et al.*, 2015; Pal *et al.*, 2018). Regarding pathology, ‘mixed dementia’ is the most common kind of dementia, affecting 46 % of individuals with

clinically confirmed AD and most frequently made up of cerebrovascular disease and AD neurodegeneration globally (Arvanitakis, Shah & Bennett, 2019). Meanwhile, less common neurodegenerative disorders include Lewy body disease, with 17 % pathologically verified cases, and frontotemporal lobar degeneration, with 5 % pathologically confirmed cases (Boyle *et al.*, 2019).

Dementia has emerged as a global issue due to the aging population, putting enormous strain on patients, their families, and health and social care organizations (Alty, Farrow & Lawler, 2020; Nunez, 2021). Delaying cognitive decline and alleviating the accompanying emotional and behavioural symptoms are the main objectives for both non-pharmacologic and pharmacologic treatments (Rahman *et al.*, 2022). In pharmacologic management, clinically and economically effective dementia medications are available, emphasizing improving or maintaining function after neuronal damage instead of altering the fundamental mechanism leading that leads to dementia state. Furthermore, drugs that are presently approved for the treatment of symptomatic dementia are acetylcholinesterase inhibitors which consist of galantamine, donepezil, and rivastigmine and N-methyl-D-aspartic acid receptor antagonists, which including memantine (Zhang *et al.*, 2020; Parsons *et al.*, 2021). Acetylcholinesterase inhibitors are now the only approved treatments for mild to moderate AD, but there is no evidence that one is more effective than the other (Haake *et al.*, 2020). However, a randomized trial recently discovered that continuous medication using donepezil is related to cognitive improvement in patients with mild to moderate dementia (Shim *et al.*, 2022).

For severe dementia symptoms, referrals to specialists like clinician managers, social workers, occupational or speech therapists, and others may be necessary (Arvanitakis, Shah & Bennett, 2019). Observational studies and randomized

controlled trials indicate that non-pharmacologic methods may be effective in treating or halting symptoms of dementia (Rolandi *et al.*, 2020; Wang *et al.*, 2020; Harris, 2021; Santagata, Massaia & D'Amelio, 2021; Islam & Adhikari, 2022). According to randomized controlled trials, cognitive training, including reading and playing cognitively challenging games like chessboard and bridge, may help keep minds sharp and bodies functioning (Rebok *et al.*, 2014). Experience-based therapies, such as music or art therapy, as well as others, may benefit cognitive preservation or quality of life (Sánchez *et al.*, 2016). Besides, both aerobic exercise, such as walking and swimming, and non-aerobic training, like weight lifting, are beneficial to cardiovascular health, and evidence from randomized controlled trials suggests that these therapeutic approaches may also enhance physical and cognitive function (Park *et al.*, 2019; Esmail *et al.*, 2020; Liu *et al.*, 2020; Raichlen *et al.*, 2020; Yu *et al.*, 2021). In addition, cognitively impaired people may also get benefit from attending social events like birthday parties and holiday gatherings, as well as attending support groups and interacting with trained animals. To improve cognition, a diet that includes nuts, leafy greens, berries, and seafood is recommended (Han *et al.*, 2017; Liu & Lai, 2017). A randomized controlled trial found that combining nutrition, cognitive exercise, and monitoring vascular risk improved cognition in people who were susceptible for cognitive decline (McCabe *et al.*, 2015). However, patients with moderate-to-severe dementia struggle to take part in any kind of activity, whether it be mental, physical, or social. The activities should be restricted when patients cannot engage effectively and safely.

### 2.2.2 Signs and Symptoms of Dementia

Pernicious, slow, but progressive dementia's manifestations are frequently confused with natural aging by older adults, families, and their caregivers (Frederiksen